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16. ABSTRACT

Seeds of Pisum sativum, varieties Little Marvel and Alaska, were planted in soils contaminated with radioactive ionic mercury, methylmercury or phenylmercury compounds. After maturation, stems, leaves, and pods were harvested and analyzed by gamma spectroscopy. Utilizing a least squares three-way analysis of covariance coupled with a Studentized Range Test, significant differences were noted among the levels of the three mercury compounds in the plants, between mercury levels in the two pea varieties and among mercury levels in the different pea tissues examined.

Phenylmercury levels differed consistently from levels of ionic mercury and methylmercury suggesting a separate pathway for it in peas.

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MOVEMENT OF MERCURY-203 IN PLANTS

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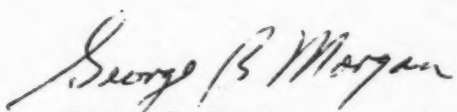
FOREWORD

Protection of the environment requires effective regulatory actions which are based on sound technical and scientific information. This information must include the quantitative description and linking of pollutant sources, transport mechanisms, interactions, and resulting effects on man and his environment. Because of the complexities involved, assessment of specific pollutants in the environment requires a total systems approach which transcends the media of air, water, and land. The Environmental Monitoring and Support Laboratory-Las Vegas contributes to the formation and enhancement of a sound integrated monitoring data base through multidisciplinary, multimedia programs designed to:

- develop and optimize systems and strategies for monitoring pollutants and their impact on the environment

- demonstrate new monitoring systems and technologies by applying them to fulfill special monitoring needs of the Agency's operating programs

Two varieties of peas were each planted in soil containing one of three radioactive forms of mercury. The total uptake and distribution of the three forms of mercury in the two pea varieties are not equal, suggesting at least two separate pathways for mercury uptake and distribution in plants. The conclusions can be beneficial in designing experiments dealing with mercury compounds and plants and also in the interpretation of data gathered by other investigators. Users who should find the report of value include the Office of Air Programs, Office of Toxic Substances, laboratories within the Office of Research and Development, other Federal agencies, and university and industrial research staffs.



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INTRODUCTION

With the findings that the toxic methylmercury compound is being formed in the environment through microorganisms-mediated transformation of inorganic mercury compounds (Bisogni and Lawrence, 1973; Jensen and Jernelov, 1969; Jernelov, 1970; Landner, 1971; McKinney, 1972; Spangler et al., 1973; Wood et al., 1968; Yamada, 1972; Yamada and Tonomura, 1972), through soil-mediated transformation of mercury compounds (Alberts et al., 1974; Kimura and Miller, 1964; Jernelov, 1970; Beckert et al., 1974) and through plant-mediated transformations of inorganic and organic mercury compounds (Fukunaga et al., 1972; Gay, 1975; Gay, in press), the investigation of plant uptake of mercury needs to be expanded to include the examination of the uptake and distribution of different mercury species in plants.

The movement and storage of mercury in a variety of plants has been determined by numerous investigators (Aarkrog and Lippert, 1971; Alvarez, 1974; Anderson and Nilsson, 1972; Barker, 1972; Bowden and McArthur, 1972; Dae et al., 1966; DeGoey et al., 1971; Fang, 1973; Findenegg and Haunold, 1972; Friedman and Waiss, 1972; Fukunaga et al., 1972; Gerdes et al., 1974; Hauskrecht and Hajduuk, 1966; Imre and Berencsi, 1972; James et al., 1971; John, 1972; Lee et al., 1972; Nardin, 1971; Pickard et al., 1963; Rao et al., 1966; Ross and Steward, 1962; Saha et al., 1970; Shacklette, 1965; Smart, 1964; Smith, 1972; Stewart and Ross, 1967; Tanner et al., 1972; Tkachuk and Kuzina, 1972; Van Loon, 1974; Yamada, 1968; Yeaple, 1972). Almost all of this work has shown the total levels of mercury in plants and plant parts via analyses by atomic absorption spectrophotometry, by neutron activation or by gamma spectroscopy using radioactive tracers. These investigations assume that the mercury compound used to treat the plant remains the same throughout the experiment.

Recent evidence suggests that this assumption is not necessarily warranted. Fukunaga et al. (1972) showed a trace amount of methylmercury in rice plants treated with various phenylmercury compounds. Gay (1975) and Gay (in press) showed that methylmercury is indeed formed in the garden pea when it is treated with ionic mercury or phenylmercury compounds through in vivo and in vitro studies. Siegel et al. (1974) have shown that a volatile form of mercury is released from plants harvested near a volcano and allowed to stand for periods of time before analysis. The mercury compound volatilized was not identified. Beauford et al. (1975) reported the release of heavy metals from plants as being associated with the loss of cuticular wax particles.

It is evident from these studies that the uptake of mercury by plants is not a simple matter. The biological pathways of the various forms need to be examined for an understanding of the potential hazard of mercury pollution to mankind via the food chain.

The present study was designed to assess the uptake, translocation and storage of three forms of mercury via root uptake from contaminated soil. Radioactive mercury-203, in the forms of mercuric acetate, methylmercuric chloride and phenylmercuric acetate, were used. The garden pea, Pisum sativum, was the plant of choice and two distinct varieties were used, a dwarf variety, Little Marvel, and a normal variety, Alaska.

CONCLUSIONS

Two varieties of the garden pea, Pisum sativum, were used. The dwarf variety, Little Marvel, accumulated and stored about twice as much mercury through root uptake from contaminated soils as the normal variety, Alaska.

Phenylmercury was accumulated and stored in the dwarf and normal varieties of peas at levels 7 and 10 times greater, respectively, than methylmercury or ionic mercury. Phenylmercury was also stored at higher levels in the pods than in the leaves or stems.

Two apparent pathways exist in peas for the movement and storage of the three mercury compounds used. Ionic and methylmercury utilize one pathway and phenylmercury utilizes another.

MATERIALS AND METHODS

Soil for the experiment was obtained from the University of Nevada Agricultural Experiment Station at Logandale, Nevada, and had the following characteristics: 54% sand, 11% clay, 35% silt, 1.3% organic carbon and pH 8.6.

Three radioactive mercury-203 compounds (mercuric acetate, methylmercuric chloride and phenylmercuric acetate) were obtained from New England Nuclear at concentrations of one millicurie each. Each isotope was handled separately to prevent any cross-contamination.

Sixty 12.7 centimeter (cm) plastic pots filled with approximately 1.3 kilograms of soil were used for each isotope. The total amount of soil needed for each isotope was sieved through 12-mesh screen into plywood boxes (91 cm in length, 61 cm in width, and 30 cm in height). The radioactive mercury compounds were each added to one liter of distilled water and the original containers were rinsed twice. Large plastic watering cans were used to slowly spray the radioactive mercury solutions onto the soil in each box while the soil was being mixed with a hoe. After the addition of the radioactive mercury solutions, it was noted that small wet dirt balls had formed throughout the soil. The soil mixture was dumped out of the boxes onto large heavy pieces of plastic and sieved again a shovelful at a time. In the sieving of each shovelful of soil, the small wet dirt balls were broken up by scraping a trowel across the screen. When all of the soil was sieved again, the box of soil was mixed again with a hoe and the 12.7 cm plastic

pots were filled. Four pea seeds were planted in each pot; 30 pots received the seeds from the dwarf variety and 30 pots received seeds of the normal variety.

To prevent cross-contamination three special exposure chambers were designed and built for each of the three radioactive mercury compounds. The chambers were made of redwood and cedar frames covered with clear acetate. All joints were sealed with 5 cm Permaseal plastic tape. The dimensions of each chamber were 137 cm in height, 152 cm in length, and 91 cm in width. A constant air flow of 1.7 m^3 min^{-1} was maintained through each chamber. The air flow was through holes in the lower edge of the long sides of each chamber, through a 12.7 cm diameter acetate plenum mounted lengthwise across the top of each chamber, through 12.7 cm stove pipe mounted down one end of each chamber with a damper control, through a prefilter and two activated charcoal filters mounted inside a fiberglass-lined plywood box and through an exhaust fan, rated at 0.235 m^3s^{-1} air flow, to the outside. Air from all three chambers converged into the box containing the filters. A platform made of redwood slats was placed 15.24 cm above the bottom of each chamber on which the pots were placed. Each pot was watered twice a day at 8 a.m. and 4 p.m. A door 61 cm by 61 cm was made in the middle of one long side of each chamber and a rubber gasket surrounded each opening. A 0.64 cm thick piece of plexiglass was used for each door and each was secured by eight bolts.

Two varieties of Pisum sativum in terms of overall growth were selected for the experiment. A dwarf variety, Little Marvel, was selected because it is a short plant displaying thick stems and large leaves. A normal variety, Alaska, was selected because it attains a height 2 to 3 times that of the dwarf and has smaller leaves and much less thickened stems than displayed by the dwarf variety.

The peas were planted December 23 and the first harvest was on February 21 with subsequent harvests on February 28 and March 7. The plants were divided into stems, leaves and pods to assess the amount of translocation and storage occurring with each form of mercury. The plants from five pots, randomly selected for each variety of pea, were harvested and pooled to make up the sample from each mercury-amended soil. Each fraction was weighed and sealed in 5 cm aluminum cans. The samples were analyzed at the Las Vegas Laboratory and the results were corrected for background and decay.

To assess significant differences among all the variables, a least squares three-way analysis of covariance statistical procedure was used. With the collection of plant samples weekly for 3 weeks, a test was needed which would determine if there were any differences in sampling over time. In the analysis of covariance test the covariant is the weekly sampling of plant parts and is represented by the regression factor.

The interactions having significant differences at the 95% confidence interval were further broken down by way of a Studentized Range Test to determine where the differences occurred.

RESULTS AND DISCUSSION

The soil-mixing technique was evaluated for thoroughness of distribution of the radioactive mercury isotopes by taking 12 random samples from each of the contaminated soils. The amount of soil used for each sample was 100 grams and the soil was sealed in 5 cm aluminum cans and analyzed by NaI (Tl) gamma spectroscopy. The 95% confidence intervals for the different contaminated soils was the mean value ±1.3% for ionic mercury, ±1.2% for phenylmercury and ±1.1% for methylmercury. The mixing technique, though simple, is extremely thorough in distributing the radioactive mercury throughout the soil.

The data realized from the experiment (see Table 1) is expressed in picocuries per gram fresh weight of tissue. The data show the two pea varieties used did not take up and store in their respective tissues, pods, leaves, and stems, equivalent amounts of the three forms of mercury added to the soil.

To determine where significant differences in mercury levels occurred a least squares three-way analysis of covariance statistical procedure was applied to the data (see Table 2). This analysis showed that significant differences exist (1) among the three forms of mercury used, (2) between the dwarf and normal varieties of peas, and (3) among selected pea tissues analyzed.

A Studentized Range Test (SRT) at the 95% probability level showing significant differences was used to determine where the significant differences occurred.

When the measured levels of the three forms of mercury used in this experiment are treated with the SRT, we find that the methylmercury level does not significantly differ overall from ionic mercury level. However, both methylmercury and ionic mercury levels differ significantly from the phenylmercury level.

When the two pea varieties are treated with the SRT, we again find overall significant differences in mercury levels between stems and leaves and stems and pods but no significant difference between leaves and pods.

Applying the SRT to the form of mercury versus the pea variety factor, we find that ionic mercury and methylmercury levels in Little Marvel or Alaska peas differ significantly from phenylmercury levels in either Little Marvel or Alaska peas and that the phenylmercury level in the Alaska peas differs significantly from the phenylmercury level in the Little Marvel peas. No significant difference was found between methylmercury and ionic mercury levels in either Little Marvel or Alaska peas. Figure 1 depicts the mean values for each form of mercury found in Alaska or Little Marvel varieties of peas.

TABLE 1. THE WET WEIGHTS AND AMOUNTS OF MERCURY-203 IN pCi/g OF SELECTED PLANT PARTS HARVESTED WEEKLY FOR THREE WEEKS FROM LITTLE MARVEL AND ALASKA PEAS

			February 21		February 28		March 7	
Hg Source	Peas	Parts	Weight (g)	pCi/g	Weight (g)	pCi/g	Weight (g)	pCi/g
Methyl	Alaska	Pods	0.69	39	1.97	44	1.24	42
		Leaves	3.74	62	6.14	51	4.25	43
		Stems	3.33	38	6.60	28	5.51	14
Methyl	Little Marvel	Pods	7.20	31	7.88	140	8.23	70
		Leaves	4.62	140	4.30	180	4.07	110
		Stems	2.00	57	1.89	130	1.38	57
Ionic	Alaska	Pods	1.46	18	3.00	39	4.77	53
		Leaves	3.01	79	5.37	65	3.17	110
		Stems	2.78	23	4.98	24	3.55	27
Ionic	Little Marvel	Pods	6.27	110	5.07	65	3.37	150
		Leaves	3.67	220	2.59	150	1.40	380
		Stems	1.57	140	1.22	74	0.87	270
Phenyl	Alaska	Pods	1.79	780	3.49	710	2.72	490
		Leaves	5.47	540	4.89	470	7.30	310
		Stems	5.48	480	5.68	370	8.02	230
Phenyl	Little Marvel	Pods	4.40	980	3.48	1200	2.73	1300
		Leaves	3.20	880	3.11	690	0.98	1100
		Stems	1.17	630	1.20	670	0.99	540

TABLE 2. LEAST SQUARES THREE-WAY ANALYSIS OF COVARIANCE

Source	DF	SS	MS	F
Form of Mercury	2	4,177,030	2,088,520	230.45*
Pea Variety	1	494,256	494,256	54.54*
Plant Parts	2	179,106	89,553	9.88*
Form of Mercury vs. Pea Variety	2	285,798	142,899	15.77*
Form of Mercury vs. Plant Parts	4	399,450	99,863	11.02*
Pea Variety vs. Plant Parts	2	17,562	8,781	0.97
Form of Mercury vs. Pea Variety vs. Plant Parts	4	42,547	10,637	1.17
Regression	1	67	67	0.007
Error	35	317,203	9,063	

*Denotes significance at the 95% confidence level.
DF = degrees of freedom
SS = sums of squares
MS = mean square
F = Fisher variance ratio

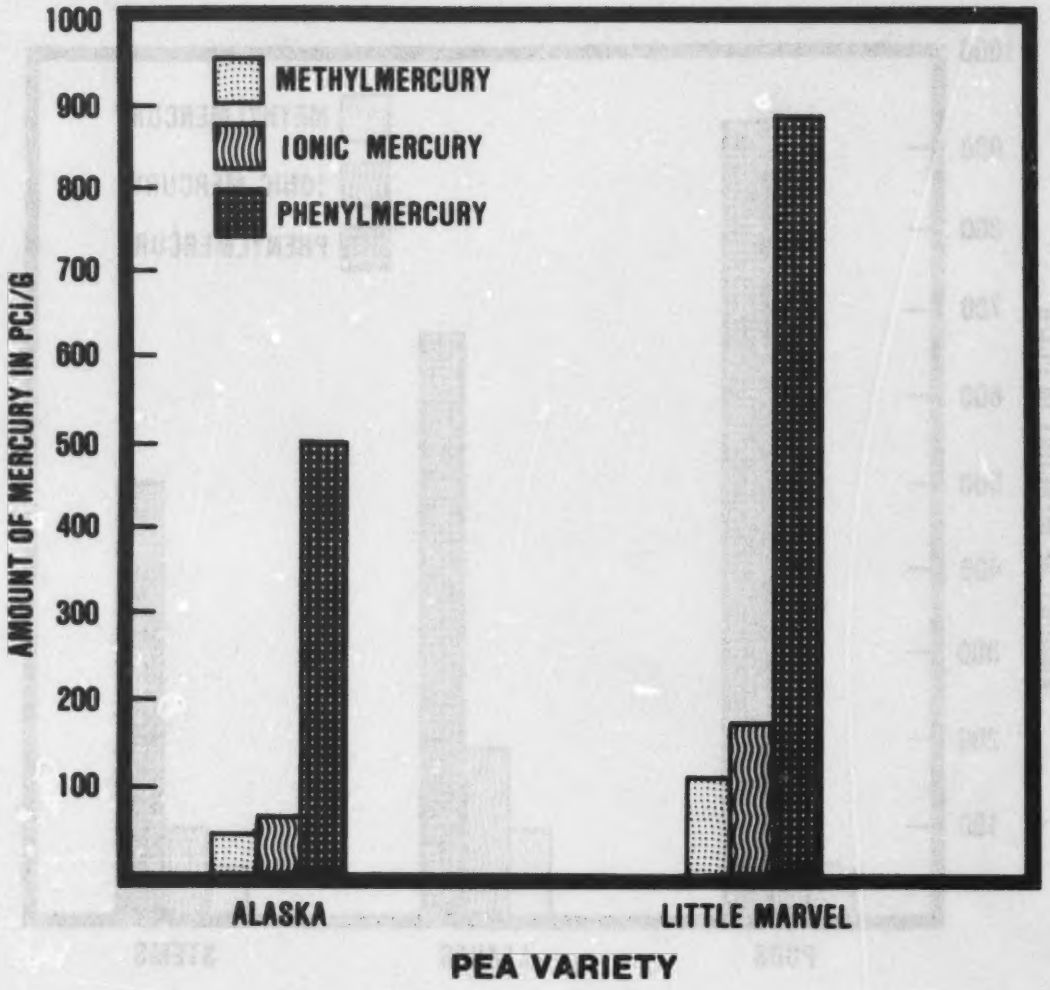

Figure 1. Mean values of the total mercury found in the Alaska and Little Marvel varieties of peas.

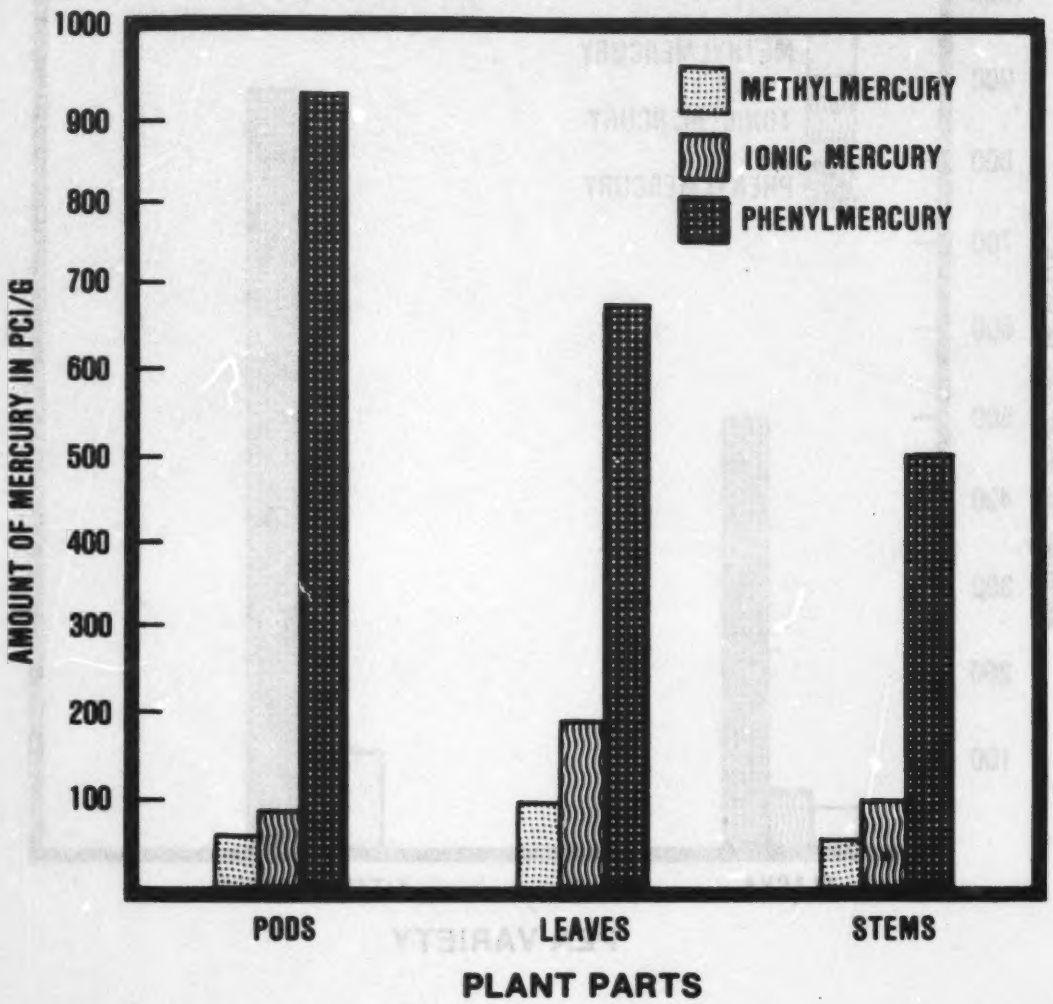

Figure 2. Mean values of the amount of mercury in various parts of the pea plant.

Looking at the interaction between the three forms of mercury and the three plant parts by way of the SRT, we find again the major difference between phenylmercury and each of the other two forms. Methylmercury in the stems, leaves or pods and ionic mercury in the stems, leaves or pods both differ significantly from phenylmercury in the stems, leaves or pods. In addition it was noted that phenylmercury in the stems and leaves differs significantly from phenylmercury in the pods.

Figure 2 depicts the mean values of the amounts of mercury found in the various plant parts. The graph shows the close relationship between methylmercury and ionic mercury and the disparity in relation of each of these to phenylmercury.

Because no significant difference was noted for pea variety versus plant parts in the analysis of covariance, the Studentized Range Test was not applied.

The results from this experiment consistently show that phenylmercury does not have the same pathway in either variety of peas as ionic or methylmercury, which utilize the same pathway.

In a previous paper (Gay, 1975), it was shown that phenylmercury and ionic mercury had different sites for their transformation to methylmercury. Although both mercurials give rise to methylmercury when incubated with plant tissues, maximum formation of methylmercury from phenylmercury occurred in the apical regions while maximum formation of methylmercury from ionic mercury occurred in subtending internodal regions. That methylmercury is formed from either mercury compound is unusual, but to have two apparent pathways for these transformations is totally an unexplored region.

This experiment supports the theory of separate but distinct pathways for the metabolism and storage of different mercury compounds in plants.

Ubiquitous mercury levels in similar plants cannot be assumed because this experiment, utilizing peas from the same genus and species but different varieties, shows that highly significant differences in mercury levels exist between two pea varieties.

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